

Case Report—

Liver Granulomas Due to *Eubacterium tortuosum* in a Seven-Week-Old Bobwhite Quail

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SUMMARY. Three 7-wk-old Bobwhite quail were submitted for necropsy to the Douglas branch of the Georgia Poultry Laboratory Network. Grossly, one bird had multiple white foci in the liver and a mild airsacculitis. In this quail there were multiple hepatic granulomas that contained mats of filamentous bacteria easily seen in hematoxylin- and eosin-stained histologic sections. These bacteria were negative with period acid-Schiff and were not acid fast. Bacteria were gram-positive but were most evident on Warthin-Starry silver-stained sections. The appearance and histochemical characteristics of these bacteria are most consistent with *Eubacterium tortuosum*.

RESUMEN. *Reporte de Caso*—Granulomas hepáticos debidos a *Eubacterium tortuosum* en una codorniz Virginiana de siete semanas de edad.

Se recibieron para necropsia tres codornices Virginianas de siete semanas de edad en el laboratorio de Douglas, Georgia, parte de la red de laboratorios de Diagnóstico del estado de Georgia. Macroscópicamente, un ave mostraba múltiples focos blancos en el hígado y una aerosaculitis leve. En esta ave se observaron múltiples granulomas hepáticos que contenían gran cantidad de bacterias filamentosas observadas fácilmente en las secciones histológicas coloreadas con hematoxilina-eosina. Estas bacterias fueron negativas a la tinción ácido resistente de Schiff. Las bacterias eran Gram positivas pero fueron más evidentes en las secciones teñidas con la coloración de plata Warthin-Starry. La apariencia y las características histoquímicas de estas bacterias son concomitantes con *Eubacterium tortuosum*.

Key words: Bobwhite quail, liver granulomas, *Eubacterium tortuosum*

Abbreviations: H&E = hematoxylin and eosin; LSFOs = long-segmented filamentous bacteria; PAS = periodic acid-Schiff; WS = Warthin-Starry silver stain

Gram-positive filamentous bacteria compatible with *Eubacterium tortuosum* have been described in granulomas observed in histologic sections of liver, spleen, and other visceral organs in chickens (5,6) and in the livers of turkeys (1,2,7). The granulomas are usually found in slaughter-age turkeys and broiler chickens at the processing plant and result in condemnation of the viscera (5,7). By light microscopy, the contorted filamentous rods of *E. tortuosum* are nonbranching and do not appear to be septate, but by electron microscopy septations are evident (2,6). In quail, *E. tortuosum* has not been reported, but there are reports of other filamentous gram-positive bacteria that were originally described as long-segmented filamentous organisms (LSFOs). These, however, are found only in the small intestine attached to the apical surface of enterocytes and do not result in granulomas in visceral organs (3). LSFOs may vary in length (up to 47 μ m long) and in width (0.7–1.2 μ m), are segmented by light microscopy, and are usually arranged in parallel arrays similar to the appearance of “railroad ties” (3). These LSFOs also have been reported in poultts experimentally infected with stunting syndrome agents (1), but their role in the production of clinical signs was unclear due to the presence of other enteric pathogens.

Histochemical staining is used to differentiate bacterial, fungal, and protozoal organisms. *E. tortuosum* are gram-positive filamentous bacteria that are negative with periodic acid-Schiff (PAS) and acid-

fast stains, but stain well with silver stains. LSFOs are gram-positive filamentous rods that are positive with PAS, stain well with silver stains, and are not acid-fast (3,4). This is the first report of granulomas in quail caused by *E. tortuosum*.

CASE REPORT

Case history and gross findings. Three 7-wk-old Bobwhite quail (*Colinus virginianus*) from a flock of 3000 were submitted for necropsy to the Georgia Poultry Laboratory Network, Douglas branch, by a private owner to monitor flock health. The flock was vaccinated for pox the previous week before submission and 3 wk prior to submission had been treated with antibiotics for airsacculitis. In one bird there were multiple white foci in the liver and a mild airsacculitis. A segment of the affected liver was fixed in 10% neutral buffered formalin, routinely processed, and hematoxylin and eosin (H&E) stained sections were examined by light microscopy. Additional histochemical stains that were applied to replicate sections after the initial microscopic examination included Brown and Brenn’s Gram stain, Warthin-Starry (WS) silver stain, PAS stain, and Ziehl-Neelsen’s acid-fast method.

Other diagnostic tests. Liver samples were submitted for virus isolation and bacterial isolation at the University of Georgia, Tifton diagnostic laboratory and Douglas branch laboratory, respectively. Virus isolation was negative, and no bacteria growth occurred on blood agar plates under aerobic conditions.

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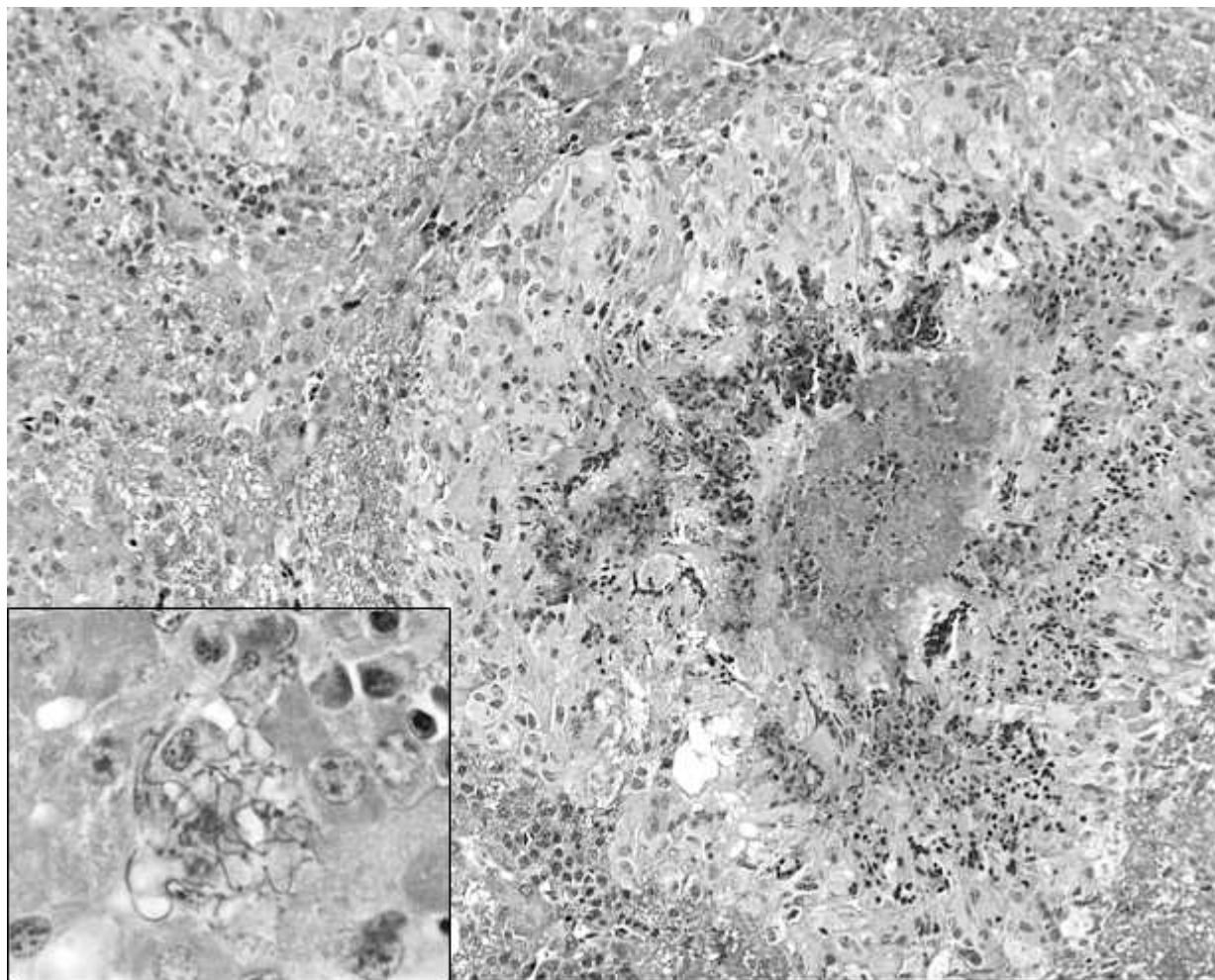


Fig. 1. Liver. Single granuloma with central necrosis and numerous filamentous bacteria. H&E 200 \times . Insert: Filamentous bacteria in sinusoids. H&E 1000 \times .

Histologic findings. In the liver numerous granulomas were characterized by accumulations of eosinophilic cellular debris surrounded by epithelioid macrophages and multinucleated giant cells. In some granulomas there also were mild to moderate numbers of lymphocytes and plasma cells peripheral to the macrophage layers with a few accompanying heterophils. Large mats of filamentous bacteria that were intensely basophilic on H&E stained sections were present within the granulomas and in the adjacent hepatic parenchyma (Fig. 1). There also were occasional aggregates of epithelioid macrophages unassociated with necrosis. The bacteria were gram-positive by Brown and Brenn's Gram stain, but the tortuous appearance was most well marked by the WS silver stain (Fig. 2). Bacteria were negative with PAS and acid-fast stains. These histochemical characteristics are consistent with those described for *E. tortuosum*.

DISCUSSION

The identification of *E. tortuosum* in turkey and broiler chicken granulomas has been previously reported (2,4,5,6,7). LSFOs have also been documented in quail exhibiting diarrhea, increased mortality, unthriftiness, and incoordination (3). Review of the literature reveals that this is the first report of granulomas in quail due to *E. tortuosum*.

Previous reports of *E. tortuosum* infections in turkeys describe the difficulty in visualizing the organisms in H&E stained sections (2), while in chickens the organisms are intensely basophilic and readily visible in H&E stains (4,5,6). In this quail the bacteria were basophilic and easily visualized, similar to those described in the reports of affected chickens. There were large mats of bacteria within granulomas, and mats of bacteria also replaced adjacent hepatocytes. When stained with WS, the organisms appeared more numerous, and the tortuous nature was easily seen under high-power magnification.

Hafner *et al.* (4) demonstrated the low pathogenicity of the *E. tortuosum* to broiler chickens by the inoculation of cultured organisms unaccompanied by other pathogens, which resulted in splenic granulomas in only a few birds. The pathogenicity in quail is also assumed to be low because of the paucity of reported disease in the literature. In this case virus isolation and aerobic bacterial culture were negative. Special media and anaerobic conditions are needed to grow *E. tortuosum* (2,4); thus the authors were unable to fulfill Koch's postulates. Arp *et al.* (2) demonstrated that combinations of *E. tortuosum* and other bacteria inoculated into poults resulted in more liver granulomas and enteric necrosis than that seen in poults inoculated solely with *E. tortuosum*. Goodwin *et al.* (3) presented three possible scenarios for the occurrence of LFSOs in poultry: normal flora, overgrowth of commensal organisms, or pathogenic

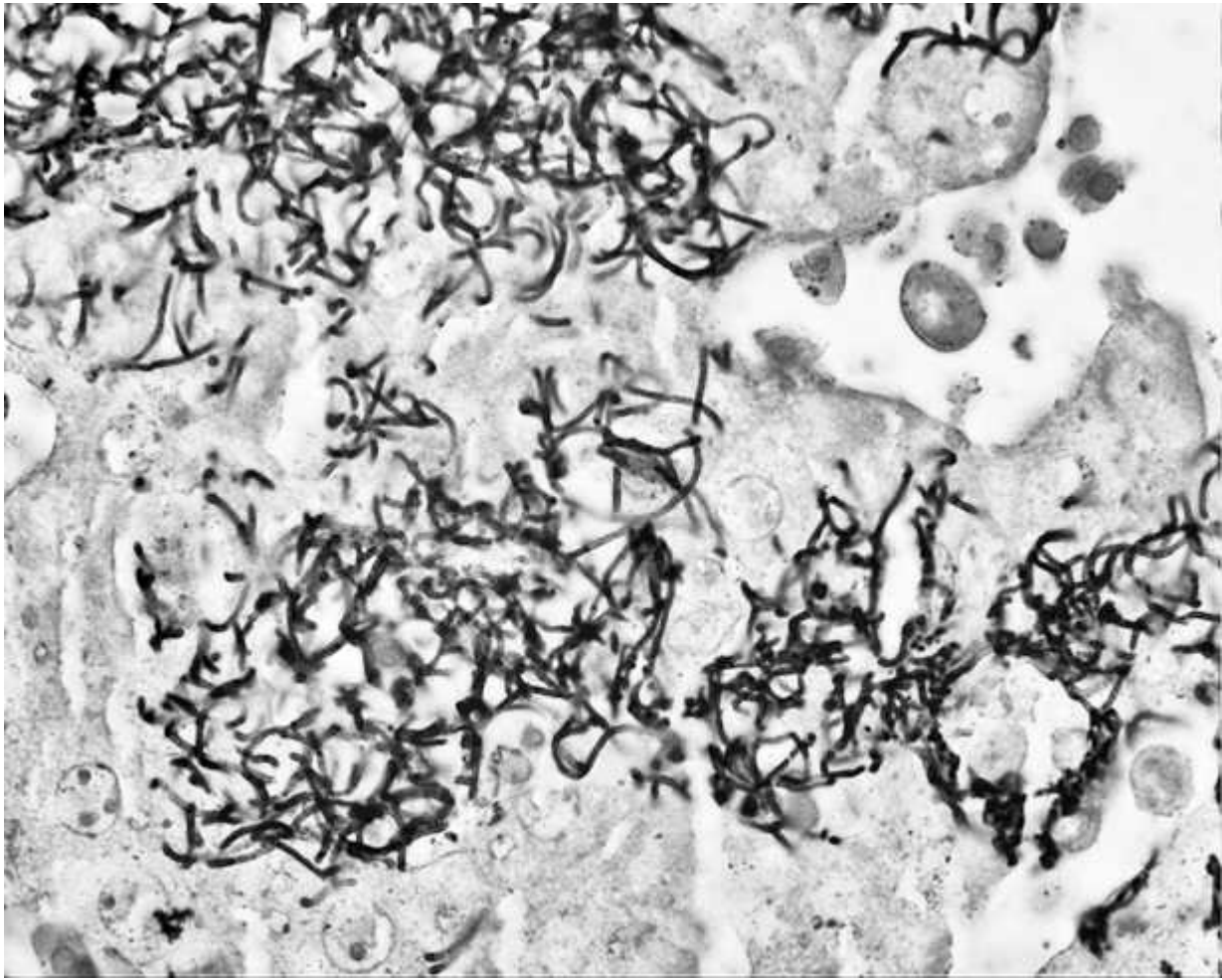


Fig. 2. Liver. Filamentous bacteria within sinusoids and hepatocytes with characteristic *E. tortuosum* appearance. WS 1000 \times .

organisms. These situations may also apply to the occurrence of *E. tortuosum* in quail. *E. tortuosum* is part of the normal cecal flora of chickens; its presence in liver granulomas in that species may be secondary to the opportunistic invasion of the intestinal wall after damage has been caused by other organisms. Histologic examination of the gastrointestinal tract for lesions was not performed in this quail; gross examination did not reveal obvious lesions. Poultry diagnosticians that provide necropsy service to quail operations or private owners should include *E. tortuosum* on the list of possible rule-outs for granulomas in visceral organs.

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